

Ca²⁺ release sites in cardiomyocytes. Since individual TT structures are below the resolution limit of conventional light microscopes, the nanoarchitecture in living heart cells is uncertain. Using STED (stimulated emission depletion) nanoscopy and the membrane probe di-8-ANEPPS, we characterized TTs quantitatively deep inside living cardiomyocytes. Using 2D analysis of TT cross-sections we determined diameters and contours indicating regular cylindrical structures. However, after myocardial infarction (post-MI) TT cross-sections appeared dilated. In addition, the continuous TT network changed progressively, resulting in loss of regular tubule orientations and sarcomere misalignment 8 weeks post-MI. In contrast, 4 weeks post-MI a significant increase of longitudinal network components was evidenced, resembling an immature embryonic phenotype. Analysis of TT associated protein expression confirmed embryonic remodeling 4 weeks post-MI. Furthermore, mathematical modeling of increased spacing between TTs and intracellular Ca²⁺ release channels revealed delayed subcellular Ca²⁺ release and action potential prolongation in heart failure. In conclusion, STED nanoscopy of intracellular TT structures in living myocytes provides direct insight about a fundamental mechanisms of excitation-contraction coupling and how TT changes contribute to Ca²⁺ release dysfunction in heart failure.

1130-Plat

Superresolution and Single Molecule Imaging of Transcription by Reflected Light Sheet Microscopy

J. Christof M. Gebhardt, Rahul Roy, David Suter, Ziqing Zhao, Alec Chapman, X. Sunney Xie.
Harvard University, Cambridge, MA, USA.

Understanding gene expression in mammalian cells requires techniques that allow to probe abundance, dynamics and distribution of the key molecular players such as transcription factors, RNA Polymerase and RNA inside the nucleus. To circumvent ensemble averaging of biomolecular dynamics, single molecule sensitivity inside the cell is essential, while superresolution imaging beyond the diffraction limit is needed to resolve the structural architecture of macromolecular assemblies involved in transcription.

We achieve both high temporal and spatial resolution in single cells using a new variant of light sheet microscopy. This new technique, Reflected Light Sheet Microscopy, employs a small mirror near the cell to project a light sheet into the nucleus. This optical sectioning reduces out-of-focus background and allows video-rate detection of individual fluorescent proteins and superresolved STORM imaging in mammalian nuclei.

In live cells, tracking of single transcription factors labeled with a fluorescent protein reveals the kinetics of free diffusion as well as non specific and specific binding to DNA. Furthermore, STORM imaging in fixed cells using fluorescently labeled antibodies against different phosphorylated forms of RNA polymerase II reveals a homogenous distribution of transcription throughout the nucleus. This superresolution view of mammalian transcription provides no evidence for transcription factories.

1131-Plat

Live-Cell Super-Resolution Fluorescence Imaging at High Spatiotemporal Resolutions

Sang-Hee Shim, Sara A. Jones, Jiang He, Xiaowei Zhuang.
Harvard University, Cambridge, MA, USA.

Super-resolution fluorescence microscopy accomplished nanometer resolutions in optical imaging along with multi-color and three-dimensional capability in fixed specimens. For live cells, time and spatial resolutions has been limited by the intrinsic tradeoff between the two resolutions. Using bright, fast switching cyanine dyes, we achieved high spatiotemporal resolutions with stochastic reconstruction microscopy (STORM). We obtained two-dimensional (2D) and three-dimensional (3D) super-resolution images of clathrin-coated pits and their transferrin cargo labeled with photoswitchable dyes either directly or via SNAP tags in living cells. The high photon output and fast switching rate of the cyanine dye enabled us to demonstrate 2D imaging at spatial resolutions of ~25 nm and temporal resolutions as fast as 0.5 sec, which represent a 2-3 fold improvement in spatial resolution and 50-100 fold improvement in temporal resolution compared to previously reported resolutions obtained using single-molecule-localization based super-resolution approaches. Furthermore, we demonstrate live-cell 3D volumetric super-resolution imaging for the first time, with a spatial resolution of ~30 nm in the lateral directions and ~50 nm in the axial direction at time resolutions down to ~1 sec. Two-color 3D super-resolution imaging in live cells was also achieved using photoswitchable probes with distinct emission spectra. In addition, we obtained similarly high spatial and temporal resolutions imaging other important cellular structures. These imaging capabilities open a new window for resolving ultrastructures in living cells.

1132-Plat

2D/3D Super-Resolution Microscopy by Means of Individual Molecule Localization (IML) and Selective Plane Illumination Microscopy

Francesca Cella Zanacchi¹, Zeno Lavagnino^{1,2}, Mario Faretta³, Laura Furia³, Alessio Del Bue¹, Michela Perrone Donnorso¹, Emiliano Ronzitti¹, Alberto Diaspro^{1,2}.

¹Italian Institute of Technology (IIT), Genoa, Italy, ²DIFI-University of Genoa, Genoa, Italy, ³Department of Experimental Oncology, IFOM-IEO Campus for Oncogenomics, Milan, Italy.

In the last few years localization based techniques, which exploit photoactivation, photoconversion or ground state depletion of fluorescent molecules, became a popular tool for super-resolution imaging of biological samples. Recently, approaches based on two photon excitation have been implemented in order to get axial confinement of the photoactivation process allowing for 3D super-resolution imaging of biological samples [1]. However, a topic of interest is still represented by the widening of super-resolution applications to thick samples (>15 µm). Within this scenario, light sheet based fluorescence microscopy techniques provide optical sectioning since illumination is confined to a thin planar region perpendicular to the detection axis and represent a suitable tool to confine the photoactivation process along the optical axis. In particular, single plane illumination microscopy (SPIM), has been proved to be a useful tool for biological investigations of thick living samples [2]. Here we demonstrate three-dimensional super-resolution live cell imaging through thick biological specimen (>50 µm), by coupling far-field individual molecule localization (IML) and selective plane illumination microscopy (SPIM). The confined excitation provided by SPIM and the improved signal-to-noise ratio allows for nanometric localization of single molecules in thick scattering samples. A PALM approach [3] and elliptical stretching of the point spread function allow to perform 3D super-resolution imaging of biological live samples in depth (up to 100 µm). IML-SPIM allowed to image cellular spheroids with < 35 nm lateral precision and sub-diffraction resolution in depth [4].

- (1) York, A.G. et al. *Nat Methods*, 8, 327–333 (2011).
- (2) Huiskens, J., et al. *Science***305**, 1007–1009 (2004).
- (3) Hess, S.T., Girirajan, T.P.K. and Mason, M.D. *Biophys J.*, 91(11), 4258–4272 (2006).
- (4) Cella Zanacchi et al. *Nature Methods* (accepted).

1133-Plat

STED - AFM: Tip Probing Enhanced by Super Resolved Targeting

Jenu V. Chacko^{1,2}, Benjamin Harke¹, Claudio Canale¹, Paolo Bianchini¹, Alberto Diaspro^{1,2}.

¹Italian Institute of Technology, Genova, Italy, ²University of Genova, Genova, Italy.

Tip based topological studies are always very dependable when we question minute structures like proteins or behaviors like aggregation, adhesion, conformational or morphological changes. Dedicated tip based microscopy, as atomic force microscopy; revealed new insights in biology and medicine in the past decades with the new ability of “touch and manipulate” objects at the nanoscale. Besides, AFM based methods solved many functional queries of the scientific community. Here, we show the enhanced ability of pointing AFM on a region of interest by super resolved imaging of a fluorescent population by coupling it to a far-field optical nanoscopy method, STED microscopy. We report about the first realization of AFM-STED hybrid architecture. We demonstrate AFM-STED measurements on various samples of scientific pursuit with a precision better than 60 nm. This new overlay of optical images of fluorescent structures to AFM surface studies; enables the system with the fundamental element of accuracy and helps in directing the tip to any region of interest. This is particularly well suited system, for example: for membrane dynamics studies and for detecting mechanically induced stress effects on precisely defined regions at the nanometer resolution. We think that this opens an important window on the design of new brand experiments in biophysics at the nanoscale.

Platform: Protein Dynamics

1134-Plat

Dynamics at Specific Sites in Proteins Studied by 2D IR Vibrational Echo Spectroscopy

Megan C. Thielges, Jean K. Chung, Michael D. Fayer.
Stanford University, Stanford, CA, USA.

The techniques of two-dimensional infrared (2D IR) spectroscopy enable the study of fluctuations of molecules and their environments on fast timescales. Characterization of specific sites in proteins requires the use of extrinsic vibration probes to alleviate the spectral complexity that hinders the application of IR spectroscopy with protein samples. Heme-bound CO has been a popular vibrational probe of protein active sites and, for example, was employed to characterize the involvement